

New Unguiphora (Platyhelminthes: Proseriata) from India

Marco Curini-Galletti, Gavino Oggiano, and Marco Casu

Dipartimento di Zoologia ed Antropologia Biologica, Università di Sassari, via Muroni 25,
I-07100 Sassari, Italy

Abstract.—Three new species of Unguiphora are described from Goa (India). Two species belong to the genus *Nematoplana* (*N. indica* and *N. calamus*). They are distinguished from the known species of the genus on the basis of the morphology of sclerotized structures. The new genus *Alloeostylyphora* is established for the third species (*A. mirabilis*), based on the unique features of its copulatory apparatus, with two symmetrical copulatory organs, and a few, non-glandular accessory stylets.

The Unguiphora is the smallest surborder of the Proseriata (Platyhelminthes). At present, the taxon is comprised of 5 genera and 34 species. In general, very few species are known from any given biogeographical area (Curini-Galletti & Martens 1991, 1992). However, the recent finding of an exceedingly rich unguiphorid fauna in eastern Australia raises the question of our actual understanding of the diversity of the taxon in extra-boreal areas (Curini-Galletti 1998, Curini-Galletti et al. 2001). The present contribution deals with three unguiphorid species found in a small sediment sample from Goa, India.

Materials and Methods

The sample (about one-half liter of sediment) was collected in intertidal pockets of clean medium-fine sand among rocks, in front of Fort Aguada Beach Hotel, Aguada, Goa (India) (May 1993). The animals were extracted from the sediment in the laboratory with the MgCl₂ decantation technique (Martens 1984). Preservation and histological techniques routinely adopted for Proseriata were used (see Martens et al. 1989); whole mounts were made with polyvinyl-lactophenol or Faure. Karyological techniques are described in Curini-Galletti et al. (1989). Idiograms (Figs. 2E, 3G) are based

on karyometrical data presented in the karyotype formula: haploid genome absolute length in μm , relative length and centromere index of each chromosome; chromosome nomenclature between parentheses (m = metacentric; sm = submetacentric). Type material is deposited in the collections of the Queensland Museum, S. Brisbane, Australia (QM).

Family Nematoplanidae Meixner, 1938
Genus *Nematoplana* Meixner, 1938
Nematoplana indica, new species
Figs. 1, 4A

Material examined.—Holotype, India, Goa, Aguada: whole mount (lactophenol) (QM-G211833).

Etymology.—Named after the geographical area, from which the species is recorded.

Description.—The holotype is an adult worm, about 4.2 mm long in fixed condition, without pigment or pigmented eye-spots. Anterior end elongate, provided laterally and terminally with sensory bristles. The pharynx, short and collar shaped, is located in the posterior fifth of the body.

Male genital organs: with numerous testes irregularly arranged between vitellaria in front of the pharynx. The copulatory organ consists of an elongate, muscular bulb

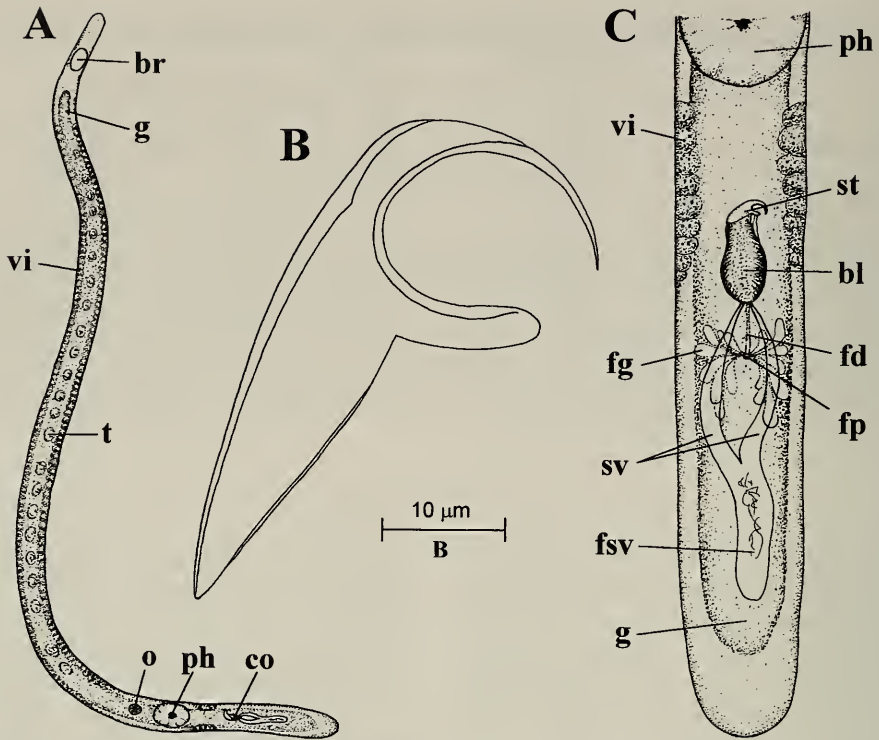


Fig. 1. *Nematoplana indica*, new species. (A) general organization of live animal; (B) stylet (from holotype); (C) detail of the post-pharyngeal area. Abbreviations in figures: ast—accessory stylet; br—brain; bl—bulb; co—copulatory organ; e—eye-spot; fd—female duct; fg—female glands; fp—female pore; fsv—fused seminal vesicles; g—gut; ma—male antrum; o—oocyte; ph—pharynx; phg—pharyngeal glands; sv—seminal vesicle; st—stylet; t—testis; vi—vitellaria.

(about 110 μm long), provided with a copulatory stylet, and two seminal vesicles, which enter the bulb proximally. The seminal vesicles, which have a very thin lining, are fused caudally. The stylet is funnel shaped, 48 μm long, with a broad, markedly oblique, proximal opening and a narrow distal opening (Fig. 1B). Its slender base is about 30 μm long; its maximum diameter (about 11 μm) is attained just below the apophysis, which is about 14 μm long and 4 μm wide. The apophysis is nearly perpendicular to the main axis of the stylet. Numerous, thick longitudinal muscles, most of which are connected basally with the musculature surrounding the bulb, are attached to the apophysis. The distal end of the stylet narrows above the apophysis into a nearly straight tube, about 0.33 of the to-

tal length of the stylet. It is provided with a long, slightly recurve, distal tip (about 20 μm long), perpendicular to the main axis of the stylet.

Female genital organs: the specimen examined possessed one mature oocyte, medially in front of the pharynx. Vitellaria extend from posterior to the brain to in front of the ovaries, and from posterior to the pharynx to the level of the copulatory bulb. The oviducts fuse posterior to the bulb into a short common female duct, which opens to the outside through a female pore, medially between the seminal vesicles.

Remarks.—The distinctly claw-shaped stylet, with an elongate base, a slightly recurve distal tip, and a long apophysis, is comparable to that of the *N. coelogyne* species group (which includes *N.*

coelogyoporoides Meixner, 1938, *N. ciliovesiculae* Tajika, 1979, *N. riegeri* Curini-Galletti & Martens, 1992 and *N. cannoni* Curini-Galletti, Oggiano & Casu, 2001) (cf. Curini-Galletti & Martens 1992, Curini-Galletti et al. 2001). The insertion of the apophysis with the axis of the stylet differs; it is perpendicular in *N. indica* and oblique (at an angle of about 45°) in the species listed above. Furthermore, the stylet of *N. indica* is straighter above the apophysis, with a much longer distal tip, that is more clearly orthogonal to the main axis, than any of the species listed above. Among the Indo-Pacific members of the *N. coelogyoporoides* group, the Japanese *N. ciliovesiculae* has a more recurve, markedly larger (about 130 µm long) stylet, with a very short distal tip and a relatively very short, obtuse apophysis. *Nematoplana cannoni* from eastern Australia is similar to *N. indica* for the size of the stylet. Furthermore, both species share the presence of unpaired oocytes, and of an elongate muscular bulb. However, in *N. cannoni* the portion of the stylet above the apophysis is recurved and comparatively longer and broader than in *N. indica*. Furthermore, its stylet is provided with a much shorter distal tip; the apophysis is oblique, narrow, and angled distally; the basis is broader proximally. *Nematoplana cannoni* has pigmented eye-spots, enclosed within the brain capsule.

Nematoplana calamus, new species
Figs. 2, 4C, D

Material examined.—Holotype: India, Goa, Aguada: whole mount (lactophenol): QM-G211834. Paratype: one karyological slide made permanent with Faure (QM-G211835), same data as holotype.

Etymology.—The name refers to the shape of the stylet, which resembles an old-fashioned pen-nib (lat. *calamus*, a noun used as an apposition).

Description.—Animals small: the holotype is an adult worm, about 1.2 mm long

in fixed condition. Without pigment or pigmented eye-spots. Anterior end elongate, provided laterally and terminally with sensory bristles. The short, collar-shaped pharynx is located in the posterior sixth of the body.

Male genital organs: with a few testes irregularly arranged among vitellaria, in front of the pharynx. The copulatory organ consists of a single seminal vesicle, and a bulb provided distally with a stylet. The seminal vesicle is broadly elongate, and provided with a coating of circular musculature. The seminal vesicle enters the bulb at its proximal base. The ovoid bulb is about 20 µm long, and is provided with numerous prostatic glands, some of which have their cell bodies outside the bulb itself. The stylet (Figs. 2B, C, 4C, D), about 18 µm long, is thin and diaphanous. Its base (about 11 µm wide) is gutter-shaped, widely open proximally and ventrally, with a marked notch at its dorsal side. The ventral sides are nearly straight, and are distinctly angled distally. The stylet is provided distally with a recurve, convex distal spike, about 10 µm long. In living specimens, the stylet appeared distinctly claw-shaped. Presumably, it acts functionally as a funnel, with the distal opening located beneath the dorsal spike, and the proximal opening formed by the adjoining of the ventral flaps.

Female genital organs: mature oocytes were not present. Vitellaria extend from behind the brain to in front of the pharynx. Female pore, surrounded by female glands, posterior to the male pore.

Karyotype: most chromosomes are metacentric ($n = 6$). Karyotype formula: 10 µm; I: 22.49, 46.36 (m); II: 18.7, 40.92 (m); III: 15.98, 44.16 (m); IV: 15.23, 29.54 (sm); V: 15.1, 43.15 (m); VI: 12.47, 43.56 (m).

Remarks.—The stylet of *Nematoplana calamus* is peculiar. In no other unguiphorid species is it gutter-shaped, gaping ventrally, and with a distal opening located beneath a dorsal spike. The presence of a single seminal vesicle is shared with *N. martensi* Curini-Galletti, Oggiano & Casu, 2001, *N. ha-*

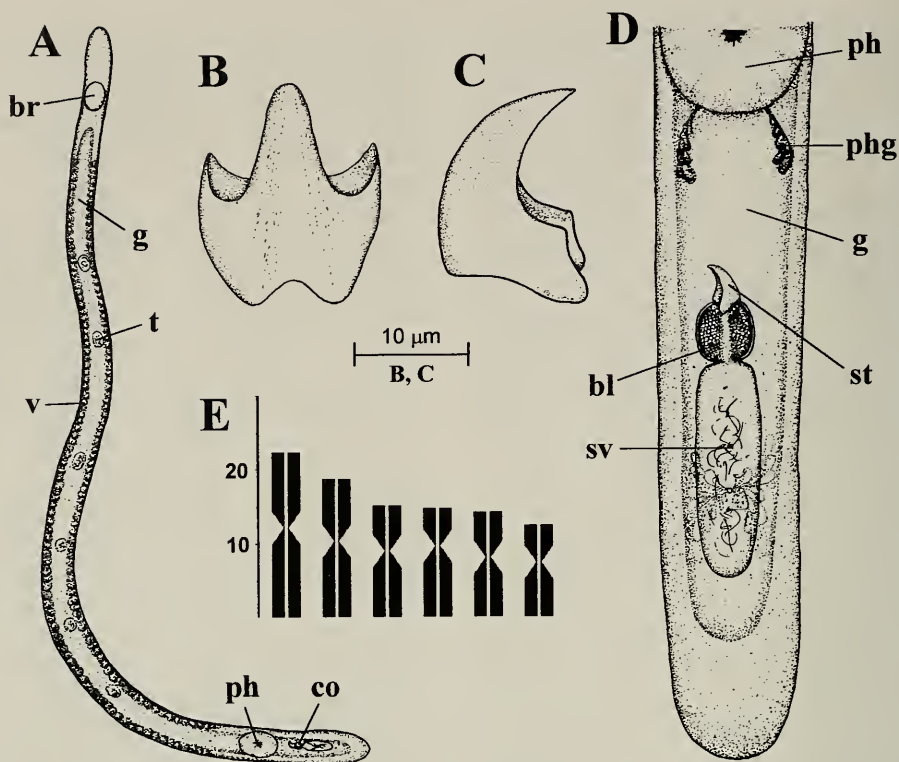


Fig. 2. *Nematoplana calamus*, new species. (A) general organization of living animal; (B, C) stylet: (B) dorsal view (from holotype); (C) lateral view (from paratype); (D) detail of post-pharyngeal area; (E) idiogram. See Fig. 1 for explanation of abbreviations.

mata Curini-Galletti, Oggiano & Casu, 2001, *N. cyclops* Curini-Galletti, Oggiano & Casu, 2001, and *N. pullolineata* Tajika, 1979 (Curini-Galletti et al. 2001, also for discussion of the problematical *N. naia* Marcus, 1949). The single-vesicle condition has been considered as a secondarily derived character status for the genus *Nematoplana*, resulting from the total fusion of the two partially fused vesicles found in the other species (Curini-Galletti & Martens 1992). This condition is not reported for any other Unguiphora. The *Nematoplana* species cited above share a further derived condition, i.e., the presence of a cylindrical stylet, not provided with an apophysis, and have been considered as constituting a monophyletic group within the genus *Nematoplana* (Curini-Galletti et al. 2001). *Nematoplana calamus* lacks any obvious

apophysis; the structure of its stylet is, however, so apomorphic that, at the moment, it precludes the inclusion of *N. calamus* in any evolutionary line known within the Unguiphora. The inclusion of *N. calamus* (as well as of the species listed immediately above, see Curini-Galletti et al., 2001) within the genus *Nematoplana* should be considered as conservative, until more data on polarization and distribution of characters are available, and a more significant fraction of the world species is described.

Alloeostylyphora, new genus

Diagnosis.—A new genus of Unguiphora with two symmetrical copulatory organs, provided with reduced bulbs. With accessory (replacement?) stylets, not connected to glandular structures. With a large male antrum anterior to the copulatory organs.

Type species.—*Alloeostylyphora mirabilis*, new species (by monotypy).

Etymology.—The generic name is coined from *alloeos*, latinized from Greek *allos* = different + *stylyphora* from the generic name *Polystylyphora* Ax, 1958. The generic name is feminine.

Alloeostylyphora mirabilis new species
Figs. 3, 4B

Material examined.—Holotype: India, Goa, Aguada: whole mount (lactophenol): QM-G211836. Paratype: one specimen sagittally sectioned (QM-G211837), same data as holotype. One immature studied karyologically.

Etymology.—*mirabilis* ('admirable') refers to the striking features of the new species.

Description.—Animals very small for the group: the holotype is about 0.8 mm long. With two pigmented eye-spots located within the brain capsule. Anterior end elongate, provided laterally and terminally with sensory bristles. Subepidermal longitudinal musculature well developed on the ventral side. Epithelium entirely ciliated (cilia length about 1 μ m), with non-insunk nuclei. The short, collar-shaped pharynx is located in the posterior fifth of the body. Its epithelium has insunk nuclei, and is ciliated except for a small area at the distal tip, where a few pharyngeal glands discharge. The cell bodies of these glands are located outside the pharynx itself. Pharyngeal cilia about 1.5 μ m long. No esophageal area could be seen.

Male genital organs: with very few (one to two), large testes among vitellaria well in front of the pharynx. With two symmetrically paired copulatory organs, posterior to the pharynx. Each consists of an elongate seminal vesicle connected to a copulatory stylet. One of the copulatory organs of the holotype had a proximally bifurcated seminal vesicle; each end was connected to a copulatory stylet. A variable number of accessory stylets (1–3) were observed, lying

very close to the copulatory stylets. In living animals, they were arranged in a row, just caudal to the copulatory stylets. These accessory stylets are not connected to the seminal vesicle, nor, apparently, to any glandular structure. The copulatory and accessory stylets are tubular structures, identical in morphology. The proximal opening is about 10 μ m wide. The dorsal side of the stylet is markedly oblique, while the ventral side is straighter, about 7 μ m long. The distal opening is about 4 μ m in diameter. The stylet is provided with a straight or slightly recurved tip, about 7 μ m long. In sections, a very small glandular area is seen, at the connection of the seminal vesicle with the copulatory stylet; this may be considered as homologous to the bulb of other species of *Unguiphora*. Each copulatory stylet protrudes into a small antrum, which opens into a broad male antrum.

Female genital organs: with only one oocyte, medially in front of the pharynx. Vitellaria extend from behind the brain to in front of the pharynx. Female pore behind the male pore.

Karyotype: chromosome 1 is appreciably larger than the other pairs ($n = 5$). Chromosomes are meta- or submetacentric. Karyotype formula: 6.5 μ m; I: 29.33, 44.92 (m); II: 19.58, 46.73 (m); III: 19.16, 46.19 (m); IV: 17.44, 36.94 (sm); V: 14.48, 36.62 (sm).

Remarks.—*Alloeostylyphora mirabilis* presents a remarkable set of features, which justifies the establishment of a new genus. Among the most striking is the fact that no other Proseriata *Unguiphora* has two functional copulatory organs. Among Proseriata *Lithophora*, *Monotoplana diorchis* Meixner, 1938 has a continuous production of copulatory organs, which are arranged into an irregular row (Meixner 1938). In *M. diorchis*, however, only the distalmost copulatory organ is functional at any time and appears to be progressively replaced by a new one. In *A. mirabilis*, the two organs are symmetrical, and both appear to be functional at the same time. The presence of two

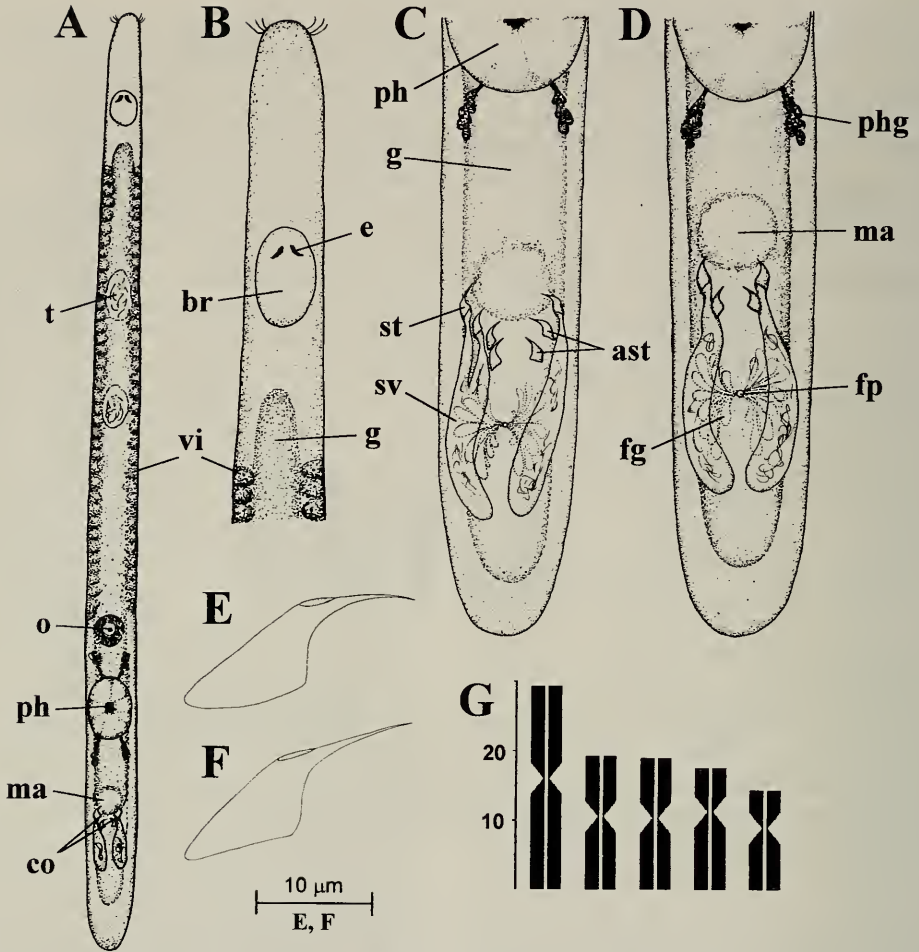


Fig. 3. *Alloeostylyphora mirabilis*, new genus, new species. (A) general organization of live animal; (B) detail of the cephalic area; (C, D) detail of post-pharyngeal area: based on holotype (C), and paratype (D); (E) copulatory stylet; (F) accessory stylet (both from holotype); (G) idiogram. See Fig. 1 for explanation of abbreviations.

seminal vesicles, connected to a copulatory bulb, is the plesiomorphic condition for the Unguiphora (Curini-Galletti & Martens 1992). The condition seen in *A. mirabilis* may thus have involved a separation of the seminal vesicles and their connection to a copulatory stylet. The presence of two copulatory organs plausibly justifies the existence of the large male antrum, similarly unknown in other Unguiphora. The extreme reduction of the copulatory bulb is a further character peculiar to *A. mirabilis*.

In addition, among Unguiphora, acces-

sory stylets are only known in the genus *Polystylyphora*, where, however, they are serially arranged, up to 55 in number, morphologically distinct from the copulatory stylet, and connected to a glandular ("prostatoid") organ (Curini-Galletti 1998, Curini-Galletti & Martens 1991). In *A. mirabilis*, copulatory and accessory stylets are morphologically identical, and the few accessory stylets present do not appear to be connected to any glandular structure. Their function is puzzling. They may act as a reservoir of functional stylets and replace the

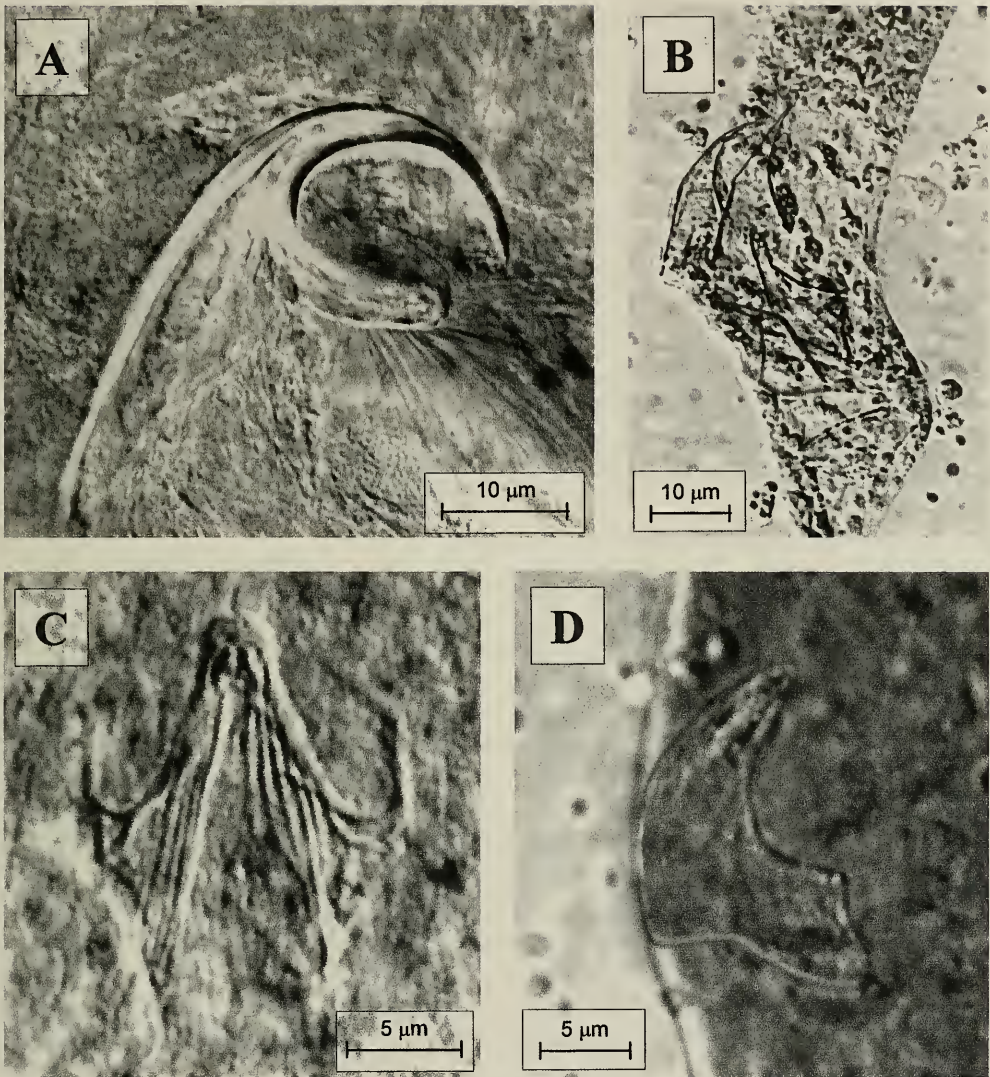


Fig. 4. (A) *Nematoplana indica*, new species, stylet (holotype). (B) *Alloeoostylyphora mirabilis*, new genus, new species, post-pharyngeal area (holotype: lactophenol whole mount, contracted after fixation). (C, D) *Nematoplana calamus*, new species, stylet: (C) from holotype; (D) from paratype.

copulatory stylet once it is damaged or discharged. Should this be the case, the holotype, which possesses one of the copulatory organs with two copulatory stylets connected to the same seminal vesicle, may thus show the transitory stage of the connection of the first accessory stylet to the seminal vesicle, before the former copulatory stylet is discharged.

The eye-spots of *Alloeoostylyphora mira-*

bilis appear identical to those found in a few species of *Nematoplana* (Curini-Galletti & Martens 1992). Their occurrence supports the hypothesis of the plesiomorphic nature of the character in the Unguiphora, proposed by Ehlers & Sopott-Ehlers 1990.

The phylogenetic relationships of *Alloeoostylyphora mirabilis* are unclear. Proposal of a sister-group relationship with the

genus *Polystylyphora* would imply the homology of the 'prostatoid stylets' with the 'accessory stylets' of *A. mirabilis*, which, at the moment, does not appear to be supported. In fact, the morphology of the 'accessory stylets' (claw-shaped, without apophysis) appears uniquely derived for the Unguiphora and is not phylogenetically informative. However, it is parsimonious to assume that the additional stylets (with a shifting of function from 'replacement' (?) to 'prostatoid', or vice versa) may have evolved only once in the Unguiphora. Findings of additional species of the group may shed light on the question.

General Considerations

Although accuracy of the morphological descriptions was in some cases hampered by the small number of specimens available for study, the sample was, nonetheless, worthy of interest due to the paucity of data from extra-European areas and for its specific and phylogenetic diversity. The presence of three unguiphorid species in a very small sample of sediment is surprising and points to a remarkable local diversity of proseriate fauna. For comparisons, the whole of northern Europe harbors only two unguiphorid species, while three species are known for the Mediterranean basin, where, however, each species occupies a distinct habitat and never co-occurs in the same sediment (Curini-Galletti & Martens 1991, 1992).

The finding of species showing unsuspected features, and whose phylogenetic relationships can not be assessed at present, clearly reflects the inadequate state of our knowledge of proseriate diversity, especially from tropical areas. A peculiar derived feature, which is apparently widespread in indo-pacific unguiphorids, but not elsewhere, is the presence of unpaired (in cases only one) oocytes in front of the pharynx (Curini-Galletti et al. 2001, present paper). There is no evidence of any further shared synapomorphies among these species, and

the character does not appear to have a phylogenetic base. A common adaptive strategy may, however, be involved. In contrast to the prevalently stocky and torpid, boreal unguiphorids, most tropical unguiphorids appear markedly smaller and more slender. Differential timing of maturity of oocytes on the two sides of the body might be due to morphological constraints of the egg size in tiny, exceedingly filiform, organisms.

Acknowledgments

We are grateful to Donatella Ciaccava Curini-Galletti, who collected and transported the sediment sample.

Literature Cited

- Ax, P., 1958. Vervielfachung des männlichen Kopulationsapparates bei Turbellarien.—Verhandlungen der Deutschen Zoologischen Gesellschaft in Graz, pp. 227–249.
- Curini-Galletti, M. 1998. The genus *Polystylyphora* Ax, 1958 (Platyhelminthes: Proseriata) in eastern Australia.—*Journal of Natural History* 32: 473–499.
- , & P. M. Martens. 1991. Systematics of the Unguiphora (Platyhelminthes: Proseriata) I. Genus *Polystylyphora* Ax, 1958.—*Journal of Natural History* 25:1089–1100.
- , & ———. 1992. Systematics of the Unguiphora (Platyhelminthes: Proseriata) II. Family Nematoplanidae Meixner, 1938.—*Journal of Natural History* 26:285–302.
- , G. Oggiano, & M. Casu. 2001. The genus *Nematoplana* Meixner, 1938 (Platyhelminthes: Unguiphora) in eastern Australia.—*Journal of Natural History* (in press).
- , I. Puccinelli, & P. M. Martens. 1989. Karyometrical analysis of 10 species of the subfamily Monocelidinae (*Proseriata*, *Platyhelminthes*) with remarks on the karyological evolution of the Monocelididae.—*Genetica* 78:169–178.
- Ehlers, U., & B. Sopott-Ehlers. 1990. Organization of Statocysts in the Otoplanidae (Plathelminthes): an ultrastructural analysis with implications for the phylogeny of the Proseriata.—*Zoomorphology* 109:309–318.
- Marcus, E., 1949. *Turbellaria Brasileiros* (7).—*Boletins da Faculdade de Filosofia, Ciencias e Letras, Universidade de S. Paulo, Zoologia*, 14:7–156.
- Martens, P. M. 1984. Comparisons of three different

- extraction methods for Turbellaria.—Marine Ecology Progress Series 14:229–283.
- , M. Curini-Galletti, & I. Puccinelli. 1989, On the morphology and karyology of the genus *Archilopsis* (Meixner) (Platyhelminthes, Proseriata).—Hydrobiologia 175:237–256.
- Meixner, J. 1938. Turbellaria (Strudelwürmer), I (Allgemeiner Teil).—Tierwelt Nordund Ostsee 33, Teil IVb: 1–146.
- Tajika, K. I., 1979. Marine Turbellarien aus Hokkaido, Japan III. Nematoplana Meixner, 1938 (Proseriata, Nematoplanidae).—Journal of the Faculty of Science of the Hokkaido University, Series VI, Zoology, 22:69–87.